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(54) Title: CRYSTALLINE SALTS OF 2-(4-{2-[2-HYDROXY-3-(2-THIOPHEN-2-YL-PHENOXY)-PROPYLAMINO]-2-METHYL-PROPYL}-PHENOXY)-NICOTINONITRILE

(57) Abstract: The present invention relates to crystalline salts of 2-(4-{2-[2-hydroxy-3-(2-thiophen-2-yl-phenoxy)-propylamino]-2-methyl-propyl}-phenoxy)-nicotinonitrile. The salts of the present invention, being  $\beta_3$  adrenergic receptor agonists, are capable of increasing lipolysis and energy expenditure in cells and, therefore, are useful, e.g., for treating type 2 diabetes and/or obesity.



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CRYSTALLINE SALTS OF 2-(4-{2-[2-HYDROXY-3-(2-THIOPHEN-2-YL-  
PHENOXY)-PROPYLAMINO]-2-METHYL-PROPYL}-PHENOXY)-  
NICOTINONITRILE

5           This application claims the benefit of U.S. Serial No. 60/292,988, filed May 23, 2001.

**Field of Invention**

10           The present invention is in the field of medicine, particularly in the treatment of type 2 diabetes and obesity. More specifically, the present invention relates to a  $\beta_3$  adrenergic receptor agonist useful in the treatment of type 2 diabetes and obesity.

**Background of the Invention**

15           The current preferred treatment for type 2, non-insulin dependent diabetes as well as obesity is diet and exercise, with a view toward weight reduction and improved insulin sensitivity. Patient compliance, however, is usually poor. The problem is compounded by the fact that there are currently no approved medications that adequately treat either type 2 diabetes or obesity.

20           One therapeutic opportunity that has recently been recognized involves the relationship between adrenergic receptor stimulation and anti-hyperglycemic effects. Compounds that act as  $\beta_3$  receptor agonists have been shown to exhibit a marked effect on lipolysis, thermogenesis and serum glucose levels in animal models of type 2 (non-insulin dependent) diabetes.

25           The  $\beta_3$  receptor, which is found in several types of human tissue including human fat tissue, has roughly 50% homology to the  $\beta_1$  and  $\beta_2$  receptor subtypes yet is considerably less abundant. Stimulation of the  $\beta_1$  and  $\beta_2$  receptors can cause adverse effects such as tachycardia, arrhythmia, or tremors. An agonist that is selective for the  $\beta_3$  receptor over the  $\beta_1$  and  $\beta_2$  receptors is, therefore, more desirable for treating type 2 diabetes or obesity relative to a non-selective agonist.

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However, recent studies have suggested the presence of an atypical beta receptor associated with atrial tachycardia in rats (*Br. J. of Pharmacol.*, 118:2085-2098, 1996). In other words, compounds that are not agonists of the  $\beta_1$  and  $\beta_2$  receptors can still modulate tachycardia through activation of a yet to be discovered  $\beta_4$  or through some other unknown pathway.

A large number of publications have appeared in recent years reporting success in discovery of agents that stimulate the  $\beta_3$  receptor. Despite these recent developments, there remains a need to develop a  $\beta_3$  receptor agonist which has minimal or no agonist activity against the  $\beta_1$  and  $\beta_2$  receptors.

### Summary of the Invention

The present invention is related to crystalline pharmaceutical acid addition salts of 2-(4-{2-[2-hydroxy-3-(2-thiophen-2-yl-phenoxy)-propylamino]-2-methyl-propyl}-phenoxy)-nicotinonitrile, hereafter referred to as SAM II.

More specifically, the present invention is related to crystalline non-solvated SAM II hemi-fumarate, hereafter referred to as the "hemi-fumarate F-I"

In addition, the present invention is related to crystalline SAM II hemi-fumarate hemi-hydrate, hereafter referred to as the "hemi-fumarate hemi-hydrate".

In addition, the present invention is related to non-solvated crystalline SAM II benzoate, hereafter referred to as the "benzoate".

In addition, the present invention is related to non-solvated crystalline SAM II (R)-mandelate, hereafter referred to as the "(R)-mandelate".

Moreover, the present invention is related to non-solvated crystalline SAM II salicylate, hereafter referred to as the "salicylate".

The present invention also relates to pharmaceutical formulations containing a crystalline salt of the present invention and a pharmaceutical carrier. In another embodiment, the pharmaceutical formulations of the present invention may be adapted for use in treating type 2 diabetes and/or obesity and/or for agonizing the  $\beta_3$  receptor.

The present invention also relates to methods for treating type 2 diabetes and/or obesity, as well as a method for agonizing the  $\beta_3$  receptor, which comprises administering to a patient in need thereof an effective amount of a salt of the present invention.

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In addition, the present invention relates to the salts of the present invention for use in treating type 2 diabetes and/or obesity as well as the salts of the present invention for use in agonizing the  $\beta_3$  receptor. The present invention is further related to the use of the salts of the present invention for the manufacture of a medicament for treating type 2 diabetes and/or obesity as well as for agonizing the  $\beta_3$  receptor.

### Brief Description of the Figures

Figure 1 is a representative XRD pattern for the hemi-fumarate F-I.

Figure 2 is a representative XRD pattern for the hemi-fumarate hemi-hydrate.

10 Figure 3 is a representative XRD pattern for the benzoate.

Figure 4 is a representative XRD pattern for the (R)-mandalate.

Figure 5 is a representative XRD pattern for the salicylate.

### Detailed Description of the Invention

#### 15 *Characterization*

Various methods, including differential thermal/thermogravimetric analysis (DT/TGA), differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), X-ray powder diffraction (XRD) and  $^{13}\text{C}$  solid state nuclear magnetic resonance (SSNMR) were used to characterize the salts of the present invention. DT/TGA is a combined system that allows for simultaneous measurement of the amount and rate of weight change (TGA) and the temperatures of endothermic and exothermic transitions (DTA). TGA is most commonly used to study desolvation processes and quantitatively determine the total volatile content of a solid. DSC is a technique that is often used to screen compounds for polymorphism because the temperatures(s) at which a physical change in a material occurs is usually characteristic of that material. DSC is often used to compliment TGA analysis in screening compounds for physical changes upon controlled heating. XRD is a technique that detects long-range order in a crystalline material.

DT/TGA was carried out on a TA simultaneous TG/DTA unit (model SDT2960). Samples were heated in open aluminum pans from 25 to 300 °C at 10 °C/min with a nitrogen purge of 150 mL/min. The temperature was calibrated with indium. The weight calibration was performed with manufacturer-supplied standards and verified against sodium tartrate desolvation.

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DT/TGA analysis of the hemi-fumarate F-I showed no weight loss prior to the onset of melting at  $\sim 170^{\circ}\text{C}$ , as expected for a non-solvated crystal form.

DT/TGA analysis of the hemi-fumarate hemi-hydrate showed a weight loss of 1.6% indicating that a stoichiometric 0.5 mole hydrate was present. An endotherm at  
5  $\sim 147^{\circ}\text{C}$  in the DTA trace represents the melt of the hemi-fumarate hemi-hydrate desolvate.

DSC analysis was also carried out on a TA Instruments Model 2910 DSC and/or Model 2920 MDSC: Model 2950 TGA and TGA analysis was carried out on a Model 2950 TGA. DSC samples were heated in aluminum pans from ambient to  $300^{\circ}\text{C}$  at  
10  $5^{\circ}\text{C}/\text{minute}$  with a nitrogen purge. TGA samples were heated in a platinum pan from ambient to  $300^{\circ}\text{C}$  with nitrogen purge.

TGA analysis of the benzoate showed no weight loss (consistent with a non-solvated crystalline form) and a melting endotherm was observed at  $149^{\circ}\text{C}$  by DSC.

TGA analysis of the (R)-mandelate shows no weight loss (consistent with a non-solvated crystalline form) and the DSC trace shows only a single sharp melting endotherm  
15 at  $\sim 138^{\circ}\text{C}$ .

TGA analysis of the salicylate shows minimal weight loss suggesting that the material is non-solvated, while the DSC trace shows a melting endotherm at  $\sim 125^{\circ}\text{C}$ .

X-ray powder diffraction patterns were obtained on a Siemens D5000 X-ray  
20 powder diffractometer which was equipped with a  $\text{CuK}\alpha$  source ( $\lambda = 1.54056$ ) operated at 50 kV and 40 mA with a Kevex solid state Si(Li) detector. The samples were scanned from  $4$  to  $35^{\circ}$  in  $2\theta$  at 3.0 seconds per step size of  $0.02^{\circ}$  with 1 mm divergence and receiving slits and a 0.1 mm detector slit. The dry powders were packed into recessed top-loading sample holders and a smooth surface was obtained using a glass slide.

Representative XRD traces of the hemi-fumarate F-I, the hemi-fumarate hemi-hydrate, benzoate, (R)-mandelate and salicylate are shown in Figures 1-5, respectively. The XRD patterns feature sharp peaks and a flat baseline, indicative of highly crystalline materials. The angular peak positions in  $2\theta$  and corresponding  $I/I_0$  data for all peaks with intensities equal to or greater than 10% of the largest peak for the hemi-fumarate F-I,  
25 benzoate and salicylate are tabulated in Tables 1, 3 and 5, respectively. The angular peak  
30 positions in  $2\theta$  and corresponding  $I/I_0$  data for all peaks with intensities equal to or

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greater than 5% of the largest peak for the hemi-fumarate hemi-hydrate and the (R)-mandelate are tabulated in Tables 2 and 4, respectively. All data in Tables 1-5 are expressed with an accuracy of  $\pm 0.1^\circ$  in  $2\theta$ .

Table 1

Angle $2\theta$	I/I <sub>o</sub> (%)	Angle $2\theta$	I/I <sub>o</sub> (%)
9.8	13.1	20.7	18.3
11.4	50.0	21.5	10.5
12.6	10.5	21.8	18.2
15.6	19.2	22.6	16.3
17.6	63.6	23.2	21.8
17.9	39.9	23.3	24.0
18.6	35.7	24.8	21.0
18.8	24.4	25.1	12.6
19.4	21.6	27.1	25.4
20.3	100	30.2	17.5

Table 2

Angle $2\theta$	I/I <sub>o</sub> (%)	Angle $2\theta$	I/I <sub>o</sub> (%)
4.2	7.9	18.6	37.5
7.8	6.4	20.6	13.1
8.4	13.6	21.3	68.7
9.9	28.9	22.9	11.9
11.4	100	23.3	13.9
12.7	51.2	23.8	14.5
15.2	19.2	25.3	6.3
15.3	13.3	26.3	5.5
16.7	6.7	27.4	11.9
17.4	7.1		

5

Table 3

Angle $2\theta$	I/I <sub>o</sub> (%)	Angle $2\theta$	I/I <sub>o</sub> (%)
5.6	29.0	26.0	41.2
7.1	36.2	26.5	14.9
8.3	26.9	26.7	12.7
8.6	100	29.8	13.5
13.4	14.7	18.7	56.5
13.7	11.8	19.3	68.3
14.9	68.0	19.9	16.9
16.1	11.6	20.4	67.2
16.7	39.2	21.5	14.8
16.8	36.2	21.8	34.6
17.2	55.4	22.2	83.5
17.6	50.9	22.4	40.2
17.8	24.8	23.3	48.6
18.0	10.3	24.2	20.1
18.4	33.4	24.7	13.0
25.6	18.3		

Table 4

Angle $2\theta$	I/I <sub>o</sub> (%)	Angle $2\theta$	I/I <sub>o</sub> (%)
4.7	100	18.6	7.6
13.2	10.2	20.0	9.0
13.4	5.6	20.5	7.8
14.5	5.9	21.1	21.7
15.3	5.2	21.8	15.1
16.9	10.0	22.3	5.2
18.2	8.3	23.5	5.7

Table 5

Angle 2 $\theta$	I/I <sub>o</sub> (%)	Angle 2 $\theta$	I/I <sub>o</sub> (%)
6.9	33.8	19.0	32.1
8.2	28.3	19.3	27.4
8.8	29.4	19.6	21.3
13.7	21.0	20.1	11.4
14.6	100	21.8	23.3
16.6	20.6	22.4	22.1
16.9	39.0	22.6	68.5
17.7	19.6	23.5	10.7
18.0	83.8	24.9	23.5

5 It is well known in the crystallography art that, for any given crystal form, the relative intensities of the diffraction peaks may vary due to preferred orientation resulting from factors such as crystal morphology and habit. Where the effects of preferred orientation are present, peak intensities are altered, but the characteristic peak positions of the polymorph are unchanged. See, *e.g.*, The United States Pharmacopeia #23, National  
10 Formulary #18, pages 1843-1844, 1995. Furthermore, it is also well known in the crystallography art that, for any given crystal form, the angular peak positions may vary slightly. For example, peak positions can shift due to a variation in the temperature at which a sample is analyzed, sample displacement, or the presence or absence of an internal standard. In the present case, a peak position variability of  $\pm 0.1^\circ$  in  $2\theta$  will take  
15 into account these potential variations without hindering the unequivocal identification of the crystalline salts of the present invention.

A well-known and accepted method for searching crystal forms in the literature is the "Fink" method. The Fink method, in general, uses the four most intense lines for the initial search followed by the next four most intense lines.

20 In general accord with the Fink method, based on peak intensities as well as peak position, the hemi-fumarate F-I may be identified by the presence of peaks at  $11.4 \pm 0.1$ ,  $17.6 \pm 0.1$ ,  $17.9 \pm 0.1$  and  $20.3 \pm 0.1^\circ$  in  $2\theta$ ; when the pattern is obtained from a copper radiation source ( $\lambda = 1.54056$ ). The presence of the hemi-fumarate F-I may be further

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verified by peaks at  $18.6 \pm 0.1$ ,  $18.8 \pm 0.1$ ,  $19.4 \pm 0.1$  and  $27.1 \pm 0.1^\circ$  in  $2\theta$ ; when the pattern is obtained from a copper radiation source ( $\lambda = 1.54056$ ).

In general accord with the Fink method, based on peak intensities as well as peak position, the hemi-fumarate hemi-hydrate may be identified by the presence of peaks at  
5  $11.4 \pm 0.1$ ,  $12.7 \pm 0.1$ ,  $18.6 \pm 0.1$  and  $21.3 \pm 0.1^\circ$  in  $2\theta$ ; when the pattern is obtained from a copper radiation source ( $\lambda = 1.54056$ ). The presence of the hemi-fumarate hemi-hydrate may be further verified by peaks at  $8.4 \pm 0.1$ ,  $9.9 \pm 0.1$ ,  $15.2 \pm 0.1$  and  $23.8 \pm 0.1^\circ$  in  $2\theta$ ; when the pattern is obtained from a copper radiation source ( $\lambda = 1.54056$ ). Peaks at  $4.2 \pm 0.1$  and  $7.8 \pm 0.1^\circ$  in  $2\theta$  are also highly indicative of the presence of the hemi-fumarate  
10 hemi-hydrate.

In accord with the Fink method, based on peak intensities as well as peak position, the benzoate may be identified by the presence of peaks at  $8.6 \pm 0.1$ ,  $14.9 \pm 0.1$ ,  $19.3 \pm 0.1$  and  $22.2 \pm 0.1^\circ$  in  $2\theta$ ; when the pattern is obtained from a copper radiation source ( $\lambda = 1.54056$ ). The presence of the benzoate may be further verified by peaks at  $17.2 \pm 0.1$ ,  
15  $17.6 \pm 0.1$ ,  $18.7 \pm 0.1$  and  $20.4 \pm 0.1^\circ$  in  $2\theta$ ; when the pattern is obtained from a copper radiation source ( $\lambda = 1.54056$ ).

In accord with the Fink method, based on peak intensities as well as peak position, the (R)-mandelate may be identified by the presence of peaks at  $4.7 \pm 0.1$ ,  $13.2 \pm 0.1$ ,  $21.1 \pm 0.1$  and  $21.8 \pm 0.1^\circ$  in  $2\theta$ ; when the pattern is obtained from a copper radiation source  
20 ( $\lambda = 1.54056$ ). The presence of the (R)-mandelate may be further verified by peaks at  $16.9 \pm 0.1$ ,  $18.2 \pm 0.1$ ,  $18.6 \pm 0.1$  and  $20.0 \pm 0.1^\circ$  in  $2\theta$ ; when the pattern is obtained from a copper radiation source ( $\lambda = 1.54056$ ).

In accord with the Fink method, based on peak intensities as well as peak position, the salicylate may be identified by the presence of peaks at  $14.6 \pm 0.1$ ,  $16.9 \pm 0.1$ ,  $18.0 \pm 0.1$  and  $22.6 \pm 0.1^\circ$  in  $2\theta$ ; when the pattern is obtained from a copper radiation source ( $\lambda = 1.54056$ ). The presence of the salicylate may be further verified by peaks at  $6.9 \pm 0.1$ ,  
25  $8.2 \pm 0.1$ ,  $8.8 \pm 0.1$  and  $19.0 \pm 0.1^\circ$  in  $2\theta$ ; when the pattern is obtained from a copper radiation source ( $\lambda = 1.54056$ ).

$^{13}\text{C}$  SSNMR analysis was performed with a Varian Unity Inova 400 MHz  
30 spectrometer operating at a carbon frequency of 100.573 MHz, using high-power proton decoupling, cross polarization (CP) and magic angle spinning (MAS) at  $\sim 7.0$  kHz.



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Acquisition parameters were as follows: 90° proton r.f. pulse width 4.0  $\mu$ s, contact time 2.5 ms, pulse repetition time 5 s, spectral width 50 kHz, and acquisition time 50 ms. Chemical shifts, expressed as parts per million, were referenced to the methyl group of hexamethylbenzene ( $\delta = 17.3$  ppm) by sample replacement. The magic angle was adjusted by optimizing the sidebands of the  $^{79}\text{Br}$  signal of KBr as described by Frye and Maciel (Frye J. S. and Maciel G. E., *J. Magn. Reson.*, **1982**, 48, 125).

The SSNMR spectrum for the hemi-fumarate F-I comprises isotropic peaks at the following chemical shifts: 19.3, 20.5, 24.5, 26.7, 40.6, 44.9, 59.5, 65.1, 66.4, 70.8, 96.5, 97.4, 113.3, 115.0, 118.4, 121.8, 123.7, 128.0, 131.3, 132.7, 133.9, 137.1, 140.4, 145.4, 150.5, 152.2, 154.8, 164.5, 172.4, 174.1 ppm.

The SSNMR spectrum for the hemi-fumarate hemi-hydrate comprises isotropic peaks at the following chemical shifts: 20.6, 22.0, 23.1, 24.3, 45.2, 59.2, 60.0, 65.2, 66.4, 70.2, 74.1, 98.4, 114.7, 116.5, 119.2, 121.8, 122.6, 127.2, 131.0, 132.7, 134.3, 134.8, 137.6, 139.8, 143.9, 151.9, 154.4, 155.4, 163.6, 173.5, 177.4 ppm.

### Synthesis

Potentiometric titrations of SAM II were performed using 0.1 M aqueous KCl medium and the measured  $\text{p}K_a$  was determined to be  $8.51 \pm 0.09$ . Therefore, the acid addition crystalline salts of the present invention are preferably those formed from reaction of SAM II with pharmaceutical acids that have  $\text{p}K_a$ 's of at least 2 units lower than the protonated amine (in order to ensure a complete acid-base reaction).

### Hemi-Fumarate F-I

The hemi-fumarate F-I may be crystallized from various organic solvents, including methanol, ethanol, isopropyl alcohol, n-propanol, acetonitrile, dimethylformamide, ethyl acetate, toluene and mixtures thereof. The hemi-fumarate F-I may also be crystallized from aqueous-organic solvent mixtures, including methanol-water, ethanol-water, isopropyl alcohol-water, acetonitrile-water, and acetone-water, when  $\leq 50\%$  water is present upon crystallization.

The hemi-fumarate F-I may also be prepared by recrystallizing the hemi-fumarate hemi-hydrate from the above-mentioned solvent systems.

*Hemi-fumarate Hemi-hydrate*

The hemi-fumarate hemi-hydrate may be prepared by recrystallizing the hemi-fumarate F-I from tetrahydrofuran-water, dimethylsulfoxide-acetone/water or  
5 dimethylformamide-ethanol/water at ambient temperature when >50% water is present at the time of nucleation. In the absence of hemi-fumarate F-I seeds, the hemi-fumarate hemi-hydrate may be prepared from ethanol and ethanol/ethyl acetate mixtures.

*Benzoate*

10 The benzoate may be isolated from single and mixed polar to moderately polar solvents (e.g., isopropyl alcohol, isopropyl alcohol-water, acetonitrile, ethanol-ethyl acetate, ethyl acetate, methyl ethyl ketone) when the temperature gradient method is employed and crystallization occurs between 25 and 50°C. For example, the benzoate may be obtained by crystallization in ethyl acetate at 50°C or a prolonged reslurry at 50°C  
15 (at a dilution which gives some solubility to the mixture). Preferred crystallization solvent systems include 9:1 ethyl acetate:ethanol, isopropyl alcohol, and acetonitrile. The benzoate salt crystallized as birefringent rods from 95% ethanol and as fine hair-like needles from the other solvents after they were allowed to evaporate.

*(R)-Mandelate*

The (*R*)-mandelate salt of the present invention may be prepared from the isomorphous ethyl acetate, isopropyl acetate, acetone, methyl ethyl ketone, ethanol and isopropyl alcohol solvates of the (*R*)-mandelate by slurrying any of said solvates in water.

Crystallization solvent systems that will produce the non-solvated form directly,  
25 via use of an (*R*)-mandelate seed, are acetone; 9% acetone/ethyl acetate (11 mL/g, 92% yield); 20% acetone/ethyl acetate (12.5 mL/g, 86% yield); and 20% isopropyl alcohol/ethyl acetate (12.5 mL/g, 92% yield).

*Salicylate*

30 The salicylate may be crystallized from 96:4 ethanol (denatured with toluene):water. Preferably, the SAM II free base starting material contains <6% total related substances (TRS), e.g., the SAM II prepared in Preparation 2 below.

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*Hemi-fumarate F-I vs. Hemi-fumarate Hemi-hydrate*

The physical stability of the hemi-fumarate F-I and the hemi-fumarate hemi-hydrate were evaluated in solution and in the solid-state as a function of temperature and relative humidity. The hemi-fumarate F-I, which melts at 176°C, is thermally more stable than the hemi-fumarate hemi-hydrate, which de-solvates above ambient temperature and subsequently melts at ~150°C. The hemi-fumarate F-I is non-hygroscopic and is thermodynamically more stable than the hemi-fumarate hemi-hydrate in all aqueous and organic media tested.

In the solid state, the hemi-fumarate hemi-hydrate is stable from 10 to 95% RH at ambient temperature but can be reversibly de-solvated above ambient temperature or upon exposure to low relative humidity at ambient temperature (~25°C).

The hemi-fumarate F-I's superior stability relative to the hemi-fumarate hemi-hydrate was demonstrated by suspending the hemi-fumarate hemi-hydrate in a solvent in which the hemi-fumarate F-I is slightly soluble and stirring in the presence of seed crystals of the hemi-fumarate F-I. Complete conversion to the hemi-fumarate F-I was observed (confirmed by X-ray diffraction and melting point) after stirring in ethanol (denatured with toluene) for 18-hours at room temperature or after 1 hour at 78°C.

The aqueous solubility for both forms varies as a function of pH, increasing with decreasing pH. The hemi-fumarate hemi-hydrate has nearly twice the solubility of the hemi-fumarate F-I at several pH's in aqueous solution. However, the solubility of both are nearly comparable in simulated intestinal fluid (fed), and enhanced relative to that in simulated intestinal fluid (fasted), suggesting that the compounds may have significant dissolution in the intestinal tract. In most organic solvents tested, the hemi-fumarate F-I is significantly less soluble than the hemi-fumarate hemi-hydrate.

The hemi-fumarate F-I gave lower exposures in both F344 rats and Cynomolgus monkeys than either the hemi-fumarate hemi-hydrate or the HCl salt. However, the exposures are acceptable to permit development of the hemi-fumarate F-I.

The hemi-fumarate F-I also has acceptable processing characteristics (granular particles which filter rapidly) and an acceptable impurity rejection (though the impurity rejection is not quite as good as the hemi-fumarate hemi-hydrate).

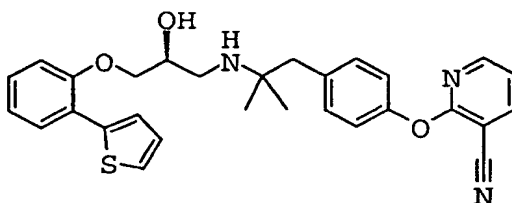
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The following examples illustrate specific procedures for preparing the crystalline salts of the present invention.

### Preparations and Examples

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#### Preparation 1: Synthesis of SAM II



A mixture of 2-(thien-2-yl)phenol (*J. Heterocycl. Chem.*, 22(6):1667-9, 1985; 1 equivalent), (2S)-glycidyl 3-nitrobenzenesulfonate (1.2 equivalent), potassium carbonate (1.2 equivalent) and acetone (8.6 ml/mol of phenol) are refluxed for 16 hours, cooled to room temperature and the solids are removed via filtration. The filtrate is concentrated and the crude product purified on silica gel (40% ethyl acetate/hexane) to give the desired epoxide.

4-(2-Amino-2-methylpropyl)phenol (50.8 g, 225 mmol), 2-chloro-3-cyanopyridine (30.8 g, 222 mmol), potassium carbonate (77.7 g, 562 mmol, powdered), N,N-dimethylacetamide (609 ml), and isooctane (122 ml) are combined and heated to reflux. The water formed during the reaction is removed azeotropically via a Dean-Stark trap. After about 1-2 hours the reaction is complete. The slurry is cooled to 30°C and filtered. The filter cake is washed with N,N-dimethylacetamide (250 ml) and the combined organic fractions are concentrated by rotary evaporation at 80°C. The resulting dark green oil is dissolved in dichloromethane (580 ml), and washed with water (160 ml). The phases are separated and the organic phase washed with water (250 ml). Water (1 L) is added to the organic phase and the pH adjusted to 1 with 12N aqueous hydrochloric acid (about 25 ml). The phases are separated and the acidic aqueous layer is washed with dichloromethane (250 ml). Dichloromethane (1 L) is added to the acidic aqueous phase and the pH is adjusted to 12-13 with 5N aqueous sodium hydroxide. The phases are

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separated and the organic phase is dried over sodium sulfate. After filtration the solution is concentrated to give 53 g of the desired amine (88%).

A stirred mixture of the epoxide (1 equivalent) and the amine (1-2 equivalents) in ethanol, methanol, n-butanol or t-butanol is heated at 70-80°C for 2-72 hours. The solvent is evaporated to dryness to give a crude oil that is optionally diluted with methanol or ethanol and passed over a cation exchange column (eluting the free base product with 1N methanolic ammonia) before further purification.

The final product may be further purified by flash or radial chromatography. Typical chromatography conditions include: a) using a variable mixture of 25:5:1 chloroform/methanol/ammonium hydroxide and 9:1 chloroform/methanol; b) a variable mixture of 90:10:1 CH<sub>2</sub>Cl<sub>2</sub>/ethanolic NH<sub>3</sub> gradient; c) dichloromethane/6-12% methanol, 0.15-0.35M ammonia in dichloromethane gradient; d) methylene chloride with a step gradient to 2-8% methanol; e) chloroform/2.0M ammonia in methanol, from 0-10% to 6-20% gradient elution or f) isocratic 6-8% 2M ammonia in methanol: 92-94% dichloromethane.

#### Preparation 2: Alternative Synthesis of SAM II

4-(2-amino-2-methylpropyl)phenol acetic acid salt (45.06 g, 200 mmol) is added to water (350 mL) and stirred at 30°C until the solid dissolves. Sodium hydroxide (5N, 41 mL, 205 mmol) is added over 5 minutes and rinsed with water (10 mL). The resulting slurry is stirred for 1 hour at 25°C followed by 45 minutes at <10°C. The product is filtered and washed with cold water (2 x 25 mL). The product is dried in a vacuum oven (50°C, 5 mmHg, 18 hours) to give 30.96 g (93.7%) of 4-(2-amino-2-methylpropyl)phenol.

Ethanol (750 mL, 2B-3), (2S)-1-(2-(thien-2-yl)phenoxy)-2,3-epoxypropane (75.0 g, 323 mmol), 4-(2-amino-2-methylpropyl)phenol (64.0 g, 387 mmol) and glacial acetic acid (485 mg, 8.07 mmol) are combined. The resulting yellow solution is stirred at 75-80°C for 18 hours until none of the epoxy starting material remained by HPLC then dimethylsulfoxide (246 mL) is added. Terephthalic acid (24.7 g, 149 mmol) is dissolved in dimethylsulfoxide (246 mL) at 60°C and then added rapidly, rinsing with 60°C dimethylsulfoxide (123 mL). The solution is seeded with (2S)-3-{[2-(4-hydroxyphenyl)-*tert*-butyl]amino}-1-(2-(2-thienyl)phenoxy)propan-2-ol terephthalate (2:1) and stirred at

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80°C for 1 hour. The white mixture is cooled to 60°C over 30 minutes and stirred for 30 minutes, cooled to 40°C over 30 minutes and stirred for 30 minutes, cooled to 20-25 °C over 30 minutes and stirred 1 hour, then filtered. The wet cake is reslurried three times in ethanol (750 mL, 2B-3) for 30 minutes then filtered. After the final filtration the cake is  
5 rinsed with ethanol (300 mL, 2B-3). After vacuum drying at 50°C/5 Torr for 12 hours, 121.2 g (77.8%) of (2S)-3-{{2-(4-Hydroxyphenyl)-*tert*-butyl}amino}-1-(2-(2-thienyl)phenoxy)propan-2-ol terephthalate (2:1) is obtained as a white solid, mp 160-162°C.

(2S)-3-{{2-(4-hydroxyphenyl)-*tert*-butyl}amino}-1-(2-(2-thienyl)phenoxy)propan-  
10 2-ol terephthalate (2:1 salt) (20.0 g, 41.4 mmol), 2-chloro-3-cyanopyridine (5.97 g, 43.1 mmol), potassium carbonate (13.73 g, 99.4 mmol) and DMSO (80 mL) are mixed. The resulting slurry is heated with stirring at 85°C until the reaction is complete (approximately 7 hours). The slurry is cooled to 30°C, Hyflow® (8 g) is added, and the mixture is stirred for 10 minutes. Ethyl acetate (140 mL) is added and the mixture is  
15 stirred for approximately 15 minutes. The mixture is filtered and the filter cake is rinsed with ethyl acetate (60 mL). The filtrate is washed sequentially with 5% NaCl solution (200 mL), 5% NaHCO<sub>3</sub> solution (200 mL), and 5% NaCl solution (2 x 200 mL). The organic layer is concentrated by rotary evaporation to give SAM II.

20 Preparation 3: Preparation of SAM II (R)-Mandelate Ethyl Acetate Solvate

SAM II is suspended in ethyl acetate and one molar equivalent R-mandelic acid is added as a powder. The slurry is heated to effect dissolution of the solids, then gradually cooled to room temperature. The solid precipitate is isolated by vacuum filtration and  
25 rinsed with ethyl acetate to give the title compound.

Preparation 4: Preparation of SAM II (R)-Mandelate Ethanolic Solvate

SAM II (253 mg) is suspended in 5 mL of ethanol denaturated with toluene and  
30 one molar equivalent R-mandelic acid (76 mg) is added as a powder. The slurry is heated to effect dissolution of the solids, then gradually cooled to room temperature and seeded with crystals of the ethyl acetate solvate of the (R)-mandelate salt. Considerable . . .

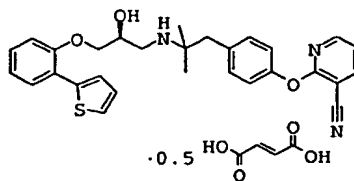
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precipitation is observed within minutes and the slurry is maintained overnight. The thick crystal slurry is diluted with 2 mL of ethanol denatured with toluene, isolated by vacuum filtration and rinsed with 3-5 mL of ethanol denatured with toluene. Yield = 250 mg of the ethanol solvate (TGA weight loss = 3.8%).

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### Example 1

#### Hemi-Fumarate F-I



10 SAM II (254 mg) is dissolved in 4 mL of absolute anhydrous ethanol at reflux (~76°C). Fumaric acid (0.5 molar equivalent, 32 mg) is added as a powder to the refluxing solution and rinsed in with 1 mL of absolute anhydrous ethanol. Solids are observed to rapidly precipitate from the refluxing solution. The crystal slurry is maintained at reflux for approximately 30 minutes, then allowed to cool to room  
15 temperature and resonate overnight. The solid product is isolated by vacuum filtration and washed with 3-5 mL of absolute anhydrous ethanol. Yield = 253 mg.

### Example 1(a)

#### Alternative Preparation of the Hemi-fumarate F-I

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The organic layer that is concentrated by rotary evaporation to give SAM II as described in Preparation 2 is concentrated to approximately 80 mL of solution and is diluted with ethanol (denatured with toluene, 120 mL). The solution is heated to 65°C with stirring and fumaric acid (2.16 g, 18.6 mmol) in ethanol (denatured with toluene, 200  
25 mL) is added. The solution is seeded and stirred at 65°C for 20 minutes. The resulting slurry is slowly cooled to room temperature over approximately 2.25 hours, then cooled to approximately 0°C for 30 minutes. The product is filtered and washed with cold 2B-3 ethanol (60 mL). The product is dried in a vacuum oven (50 °C, 5 mmHg, 18 h) to give 19.52 g (86.1%) of the title compound as a white solid, mp 172.5-174°C.

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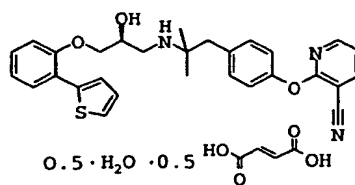
Example 1(b)

## Alternative Preparation of the Hemi-fumarate F-I

- 5        The hemifumarate hemihydrate (100 mg) is suspended in 3 mL of absolute anhydrous ethanol, and the slurry is heated to reflux. Incomplete dissolution is observed, so an additional 2 mL absolute anhydrous ethanol is added. The slurry is maintained at reflux for about 15 minutes and then allowed to cool to room temperature. The solids are isolated by vacuum filtration and washed with a few mL of absolute anhydrous ethanol.
- 10    Yield = 87 mg.

Example 2

## Hemi-Fumarate Hemi-Hydrate



- 15        SAM II (250 mg) and 0.5 molar equivalents (29 mg) of fumaric acid are suspended in 5 mL of ethanol denatured with toluene and the mixture is heated mildly to effect dissolution. After approximately five minutes, the solution begins to precipitate. The temperature of the crystal slurry is maintained at the crystallization temperature (56-
- 20    57°C) for about one hour. The heat source is then turned off and the slurry is allowed to cool with stirring overnight. Ethanol denatured with toluene (2 mL) is added and the solids are isolated by vacuum filtration. The filter cake is washed with ethanol denatured with toluene (5 mL) and air dried to give 230 mg of the title compound. mp = 147-149°C (measured by differential scanning calorimetry (DSC) with a scan rate of 10°C/minute).
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### Example 2(a)

### Alternative Synthesis of the Hemi-Fumarate Hemi-Hydrate

The hemi-fumarate hemi-hydrate is prepared by dissolving the hemi-fumarate F-I (101 mg) in 1:1 v/v tetrahydrofuran-water (3.5 mL) and adding water (10 mL) dropwise at ambient temperature. The solution becomes milky with minimal water addition and a solid precipitate is observed after addition of ~5 mL water. The solid product is immediately isolated by vacuum filtration and washed with water. Yield = 82 mg of hairs/rods.

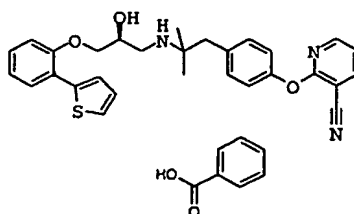
### Example 2(b)

### Alternative Synthesis of the Hemi-Fumarate Hemi-Hydrate

The hemi-fumarate hemi-hydrate is prepared by dissolving the hemifumarate F-I (101 mg) in 1:1 v/v dimethylsulfoxide-acetone (4 mL) and adding water (20 mL) dropwise at ambient temperature. Approximately 9 mL water is required to effect precipitation. The solids are immediately isolated by vacuum filtration and washed with water. Yield = 92 mg, clusters of rods.

### Example 3

## The Benzoate



SAM II (57.7 mg) is dissolved in 2.5 mL of absolute ethanol and the solution is stirred at room temperature. To the stirred solution is added benzoic acid (1 equivalent, 14.1 mg) dissolved in 200 microliters of methanol. The resulting mixture is stirred at room temperature for 3.5 to 4 hours. Precipitation occurs in approximately 30-60 minutes. The precipitate is isolated by vacuum filtration and the filter cake is collected

and air-dried overnight. mp = 148-150°C (measured by DSC with a scan rate of 5°C/minute).

### Example 3(a)

### Alternative Preparation of the Benzoate

Benzoic acid (3.66 g, 30 mmol) is added to a 50°C solution of SAM II (15.0 g, 30 mmol) in ethyl acetate (300 mL, 20 mL/g freebase). The homogeneous solution is seeded and after approximately 5 minutes, nucleation occurred. The mixture is held at 50°C for 4 hours during the precipitation. After cooling to room temperature (27°C), the mixture is filtered and the solid is washed with ethyl acetate (45 mL). The product is air-dried for 30 minutes to give 11.6 g (62% yield) of white, hair-like needles. The solid is vacuum-dried at 50°C/5 Torr with no additional loss of weight.

### Example 3(b)

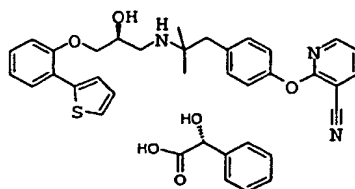
### Alternative Preparation of the Benzoate

Solid benzoic acid (1.22 g, 10.0 mmol) is added to an approximately 60°C solution of SAM II (5.00 g, 90.7% pure, 9.07 mmol) in 90:10 ethyl acetate:ethanol (100 mL). The solution is seeded with a small amount of the title compound. The solution is allowed to cool to approximately 47°C and stir for 2-3 hours while the product precipitates. The slurry is cooled to room temperature and filtered. The filter cake is washed with cold ethyl acetate (20 mL) and dried in a vacuum oven overnight at 50°C to give 4.05 g (71.8% yield, 99.1% purity) of the title compound as a white solid, mp 149.8°C (by DSC).

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Example 4

## The (R)-Mandelate



5 SAM II (200 mg) is dissolved in 1 mL of acetone and the solution is stirred at room temperature. To the stirred solution is added R-mandelic acid (1 equivalent, 61 mg) in acetone (1 ml). The resulting mixture is stirred at room temperature and the precipitate is isolated by vacuum filtration. The filter cake is collected and air-dried overnight. mp = 138-140 °C (measured by DSC with a scan rate of 5°C/minute).

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Example 4(a)

## Alternative Preparation of the (R)-Mandelate

A solution of (*R*)-mandelic acid (6.09 g, 40.0 mmol) in acetone (30 mL) is added  
15 to a solution of SAM II (20.0 g, 40.0 mmol) in acetone (100 mL, 5.0 mL/g freebase) at 57°C. The homogeneous solution is heated at reflux for 5 minutes and then slowly cooled. The solution is seeded at 44.3°C. Crystallization began at 41°C. After cooling to room temperature, the mixture is filtered and the filter cake is washed with room temperature acetone (10 mL). The solid is air-dried for 15 minutes and vacuum-dried  
20 overnight at 50°C/5 Torr to afford 22.09 g (84.7% yield) of white solid.

The solid and filtrate are recombined, diluted with acetone to a total volume of 130 mL, heated to 56°C, and the solution is filtered to remove a few fine particles. The solution is cooled very slowly while seeding repeatedly with at 50°C, 48°C, and 47°C, at which point precipitation began to occur. The mixture is held at 47°C for 45 minutes,  
25 held at 45°C for 30 minutes, and is then cooled at a rate of 2.5°C every 15 minutes until reaching 25°C. The mixture is filtered but the solid is not washed. Instead, the solid is air-dried in the filter overnight and is then vacuum-dried at 50°C/5 Torr to give 17.1 g of white crystalline product (85% yield, 0.2% TRS).

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Example 4(b)

## Alternative Preparation of the (R)-Mandelate

To a solution of R-(-)-mandelic acid (0.3 g, 2.00 mmol) in ethyl acetate (3 mL) at  
5 temperature 50°C is added SAM II (1 g, 2 mmol) dissolved in ethyl acetate (4 mL). The  
flask is rinsed with ethyl acetate (3 mL). Acetone (1 mL) is added. The solution is heated  
to a reflux, seeded, then cooled in 5 °C increments. Once crystal formation starts, the  
solution is held at that temperature for one hour, then cooled at a rate of 5°C every 30  
minutes until at 2° C. The solid is collected by vacuum filtration at 40°C under vacuum  
10 for two days.

Alternatively, Experiment 4(b) may be performed with an initial reaction  
temperature of 40°C. Under these conditions, 2.5 ml (instead of 1 mL) of acetone is  
used.

Alternatively, Experiment 4(b) may be performed with an initial reaction  
15 temperature of 65°C. Under these conditions, isopropyl alcohol is used in place of ethyl  
acetate and 2.5 ml (instead of 1 mL) of acetone is used.

Alternatively, Experiment 4(b) may be performed using acetonitrile in place of  
ethyl acetate. Under these conditions, the addition of acetone is not necessary.

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Example 4(c)

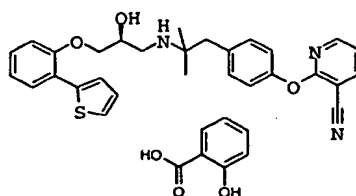
## Alternative Preparation of the (R)-Mandelate

The compound of Preparation 4 (200 mg) is slurried in 5 mL of water at ambient  
temperature for 28 hours. The solids are then isolated by vacuum filtration, washed with  
25 2-3 mL water, dried under an air stream for 30 minutes and then vacuum dried at 55°C for  
about 2 hours. m.p. = 138°C.

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Example 5

## The Salicylate



5 SAM II (106 mg) is dissolved in 1 mL of ethyl acetate and the solution is stirred at room temperature. To the stirred solution is added salicylic acid (1 equivalent, 29 mg) in 150 microliters of methanol. The resulting mixture is stirred at room temperature and then heated up 50°C. Hexane is added to the mixture at elevated temperature until cloud point (approximately 1 ml ethyl acetate:1 ml of hexane). The slurry is allowed to slowly  
10 cool to room temperature. The precipitate is isolated by vacuum filtration and the filter cake is collected and air dried overnight. mp = 124 °C (measured by DSC with a scan rate of 5°C/minute).

Example 5(a)

## Alternative Preparation of the Salicylate

15 A solution of salicylic acid (4.15 g, 30 mmol) in 96:4 ethanol/water (25 mL) is added to a solution of SAM II (15.0 g, 30 mmol) in 96:4 ethanol (denatured with toluene)/water (75 mL, 5.0 mL/g freebase) at 75 °C. The homogeneous solution is cooled  
20 slowly and seeded every 5°C. The seeds remained undissolved at 50-55 °C and nucleation occurred at 50°C. The temperature is held at 45-50 °C for 1 hour while the product precipitates. The mixture became thick and difficult to stir magnetically so an additional 25 mL of warm 96:4 ethanol denatured with toluene/water is added to facilitate stirring. After 15 min, the heating mantle is removed and the mixture is allowed to cool  
25 slowly to approximately 30°C. The mixture is filtered, the filter cake is washed with ethanol denatured with toluene (15 mL), and the solid is air-dried to give 14.97 g (78% yield) of a fluffy white solid. This material is vacuum-dried overnight at 50°C/5 Torr to give 14.46 g (0.2% w/w loss on drying) of a fluffy white solid.

Formulation

The salts of the present invention are preferably formulated in a unit dosage form prior to administration to the recipient patient. The term "patient" includes human and non-human animals such as companion animals (dogs and cats and the like). Therefore, yet another embodiment of the present invention is a pharmaceutical composition comprising a salt of the present invention and a pharmaceutical carrier. The term "pharmaceutical" when used herein as an adjective means substantially non-deleterious to the recipient patient.

The present pharmaceutical formulations are prepared by known procedures using well-known and readily available ingredients. In making the formulations of the present invention, the active ingredient (a crystalline salt of the present invention) will usually be mixed with a carrier, or diluted by a carrier, or enclosed within a carrier which may be in the form of a capsule, sachet, paper or other container. When the carrier serves as a diluent, it may be a solid, semisolid or liquid material that acts as a vehicle, excipient or medium for the active ingredient. Thus, the compositions can be in the form of tablets, pills, powders, lozenges, sachets, cachets, elixirs, suspensions, emulsions, solutions, syrups, aerosol (as a solid or in a liquid medium), soft and hard gelatin capsules, suppositories, sterile injectable solutions and sterile packaged powders.

Some examples of suitable carriers, excipients, and diluents include lactose, dextrose, sucrose, sorbitol, mannitol, starches, gum acacia, calcium phosphate, alginates, tragacanth, gelatin, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, water syrup, methyl cellulose, methyl and propylhydroxybenzoates, talc, magnesium stearate and mineral oil. The formulations can additionally include lubricating agents, wetting agents, emulsifying and suspending agents, preserving agents, sweetening agents or flavoring agents. The compositions of the invention may be formulated so as to provide quick, sustained or delayed release of the active ingredient after administration to the recipient patient.

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Formulation Example 1: 5mg Capsule

Ingredient	Quantity (mg/capsule)
Hemi-fumarate F-I	5.6
Mannitol	177.2-201.2
Microcrystalline Cellulose	177.2-201.2
Povidone	8.0-16.0
Sodium Lauryl Sulfate	1.0-3.0
Magnesium Stearate	1.0-3.0

Formulation Example 2: 25mg Capsule

Ingredient	Quantity (mg/capsule)
Hemi-fumarate F-I	28.2
Mannitol	170.4-195.0
Microcrystalline Cellulose	170.4-195.0
Povidone	8.2-16.4
Sodium Lauryl Sulfate	1.0-3.1
Magnesium Stearate	1.0-3.1

The capsules above are manufactured by an aqueous granulation process. The mannitol, microcrystalline cellulose, and active ingredient are added to the granulator and dry blended for a suitable period of time to uniformly distribute the powders. A previously prepared granulation solution consisting of purified water, povidone, and sodium lauryl sulfate is sprayed at a uniform rate onto the powders while mixing. When a suitable granulation endpoint is reached, the granulator is stopped and the granulation is unloaded. The granulation is then wet sieved, through a suitable screen to disrupt large agglomerates, spread on paper lined trays, and dried in a convection oven until the moisture is reduced to a suitable level. The size of the granulation is adjusted to a range consistent with automated capsule filling equipment requirements by passing through a co-mill or other suitable apparatus. These powders are collected and transferred to a mixing apparatus with a specified quantity of magnesium stearate. The entire powder mixture is blended for a suitable length of time to uniformly distribute the lubricant. The finished powders are then filled into hard gelatin capsules on a suitable piece of automated capsule filling equipment. Following the filling operation, the finished capsules are visually inspected for external defects. To improve the pharmaceutical elegance of the finished product, the capsules may be physically de-dusted and polished by either manual or automated processes.

Demonstration of Function

The genes encoding the human  $\beta_1$ -adrenergic receptor (Friele *et al.*, *Proc. Natl. Acad. Sci.*, 84:7920-7924, 1987), the human  $\beta_2$ -adrenergic receptor (Kobika *et al.*, *Proc. Natl. Acad. Sci.*, 84:46-50, 1987, Emorine *et al.*, *Proc. Natl. Acad. Sci.*, 84:6995-6999,

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1987) and the human  $\beta_3$  adrenergic receptor (Granneman *et al.*, *Molecular Pharmacology*, 44(2):264-70, 1993) are individually subcloned into a phd expression vector (Grinnell *et al.*, *Bio/Technology*, 5:1189-1192, 1987) and transfected into the DXB-11 Chinese hamster ovary (CHO) cell line by calcium phosphate precipitation methodology. The stably transfected cells are grown to 95% confluency in 95% Dulbecco's modified Eagles Medium (DMEM), 5% fetal bovine serum and 0.01% proline. Media is removed and the cells are washed with phosphate buffered (pH 7.4) saline (without magnesium and calcium). Cells are then lifted using an enzyme free cell dissociation solution (Specialty Media, Lavallette, New Jersey) and pelleted by centrifugation.

Cells from each of the above cell lines are resuspended and added (20,000/well) to a 96-well plate. Cells are incubated at 37°C with representative compounds of the invention for 20 minutes in buffer (Hank's balanced salt solution, 10 mM HEPES, 0.1% BSA, 1 mM L-ascorbic acid, 0.2% dimethyl sulfoxide, 1 mM 3-isobutyl-1-methylxanthine, pH 7.4). After halting the incubation with quench buffer (50 mM Na Acetate, 0.25% Triton X-100, pH 5.8), the c-AMP level is quantified by scintillation proximity assay (SPA) using a modification of the commercially available c-AMP kit (Amersham, Arlington Heights, IL) with rabbit anti-cAMP antibody (ICN Biomedicals, Aurora, Ohio) for the kit.

Sigmoidal dose response curves, from the whole cell receptor coupled c-AMP assay are fit to a four parameter logistic equation using non linear regression:  $y=(a-d)/(1+(Dose/c)^b)+d$  where a and d are responses at zero and maximal dose, b is the slope factor and c is the  $EC_{50}$  as previously described (DeLean *et al.*, *Am. J. Physiol.*, 235, E97-E102, 1978).  $EC_{50}$  is assessed as the concentration producing 50% of the maximum response to each agonist.

Isoproterenol is accepted in the art as a non-selective  $\beta_3$  agonist and is widely used as a comparator in evaluating the activity of compounds. See *Trends in Pharm. Sci.*, 15:3, 1994. The % intrinsic activity ( $E_{max}$ ) of representative salts of the present invention were assessed relative to isoproterenol by the compound's maximal response divided by the isoproterenol maximal response times 100.  $E_{max}$  and Standard Error Mean (SEM) data generated for these salts is presented below in Table 6. The  $E_{max}$  values represent the average of three runs.



Table 6

Salt	E <sub>max</sub> (SEM)
Hemi-fumarate hemi-hydrate	85.8 (6.3)
Benzoate	83.6 (3.74)
(R)-Mandalate	86.4 (0.93)
Salicylate	97.6 (5.78)

*In vitro* Rat Atrial Tachycardia

5 Male Sprague-Dawley rats (250-400 grams) (Harlan Sprague-Dawley, Indianapolis, Indiana USA) are killed by cervical dislocation. Hearts are removed and the left and right atria are dissected and mounted with thread in tissue baths containing 10 ml of modified Krebs' solution of the following composition (mM concentrations): NaCl 118.2, KCl 4.6, CaCl<sub>2</sub> · 2H<sub>2</sub>O 1.6, KH<sub>2</sub>PO<sub>4</sub> 1.2, glucose 10.0, NaHCO<sub>3</sub> 24.8. An initial  
10 resting force of 1 gram is applied to the atria (Cohen et al, *Naunyn-Schmiedeberg's Arch. Pharmacol.*, 320:145-152, 1982). Tissues are allowed to equilibrate approximately 30 minutes with vigorous oxygenation before exposure to drugs. Concentrations of SAM II dissolved in polyethylene glycol 300 and saline, are added cumulatively every 4-5 minutes. SAM II addition is continued until no further increase in atrial rate occurred or  
15 until a concentration of 10<sup>-4</sup>M is reached. The increase in beats per minute (bpm) is measured with Sensotec transducers (model MBL-5514-02) for each concentration of SAM II and recorded by a Biopac Data Acquisition System. Spontaneously beating rat atria received either vehicle or SAM II.

SAM II did not increase heart rate (n=3; 1.8 ± 0.07% increase above starting heart rate) relative to vehicle (n=8; 3.5 ± 0.6% increase in heart rate above starting heart rate).  
20 No significant difference is observed in the initial heart rates in the two groups studied. These results are expressed as mean ± the standard error of the mean where n represents the number of isolated atria examined. These data are expressed as a percent of the basal increase in heart rate and as a change in heart rate in beats per minute (bpm) from basal  
25 heart rates.

Utilities

As agonists of the  $\beta_3$  receptor, the salts of the present invention are useful in treating conditions in human and non-human animals in which the  $\beta_3$  receptor has been demonstrated to play a role. The terms "treating" and "treat", as used herein, include their generally accepted meanings, *i.e.*, alleviating, ameliorating, managing, preventing, prohibiting, restraining, slowing, stopping, or reversing the progression or severity of a pathological condition, or sequela thereof, described herein. The term "preventing" refers to reducing the likelihood that the recipient patient of a salt of the present invention will incur or develop any of the pathological conditions, or sequela thereof, described herein.

The diseases, disorders or conditions for which compounds of the present invention are useful in treating include, but are not limited to, (1) diabetes mellitus, (2) hyperglycemia, (3) obesity, (4) hyperlipidemia, (5) hypertriglyceridemia, (6) hypercholesterolemia, (7) atherosclerosis of coronary, cerebrovascular and peripheral arteries, (8) hypertension, (9) disorders of the gall bladder including acute and chronic cholecystitis, (10) depression, (11) elevated intra-ocular pressure and glaucoma, (12) non-specific diarrhea dumping syndrome, (13) hepatic steatosis [fatty degeneration of the liver], and obesity dependent diseases/disorders such as: (14) gastrointestinal disorders including peptid ulcer, esophagitis, gastritis and duodenitis, (including that induced by *H. pylori*), intestinal ulcerations (including inflammatory bowel disease, ulcerative colitis, Crohn's disease and proctitis) and gastrointestinal ulcerations, (15) irritable bowel syndrome and other disorders needing decreased gut motility, (16) diabetic retinopathy, (17) neuropathic bladder dysfunction, (18) osteoarthritis, (19) restrictive lung disease, (20) obstructive sleep apnea, (21) congestive heart failure, (22) venous stasis and skin disorders related to venous stasis, (23) decreased libido (in both males and females), and (24) acute and chronic cystitis. The term "obesity dependent" means that the symptoms of said diseases will be ameliorated via the present salt's effect on the patient's weight.

Human patients in need of obesity treatment are typically those with a body mass index (BMI)  $>27$  or those with a BMI  $\geq 25$  when co-morbidities, *e.g.*, hypertension, sleep apnea and/or osteoarthritis, are present. A patient population at particular need of treatment are those with a BMI  $>30$  or  $>27$  with co-morbidities.

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Human patients in need of hypertension treatment are frequently overweight individuals, i.e., those with a BMI  $\geq 25$ , but may also be of normal body weight (i.e., BMI  $< 25$ ).

Human patients in need of type 2 diabetes treatment are typically individuals with  
5 a BMI  $< 25$ , i.e., individuals that are not overweight.

### Dose

As used herein, the term "effective amount" means an amount of a salt of the present invention that is capable of treating the conditions described herein or that is  
10 capable of agonizing the  $\beta_3$  receptor.

The specific dose or amount administered is determined by the particular circumstances surrounding each situation. These circumstances include, the route of administration, the prior medical history of the recipient, the pathological condition or symptom being treated, the severity of the condition/symptom being treated, and the age  
15 and sex of the recipient patient. However, it will be understood that the therapeutic dosage administered will be determined by the physician in the light of the relevant circumstances.

Generally, an effective minimum daily dose of a salt of the present invention will exceed about 5 mg. Typically, an effective maximum daily dose will not exceed about  
20 350 mg. The exact dose may be determined, in accordance with the standard practice in the medical arts of "dose titrating" the recipient; that is, initially administering a low dose of the compound, and gradually increasing the dose until the desired therapeutic effect is observed.

### 25 Route of Administration

The crystalline salts of the present invention can be administered by a variety of routes including the oral, rectal, transdermal, subcutaneous, topical, intravenous, intramuscular or intranasal routes. A preferred route of administration is the oral route.

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Combination Therapy

The crystalline salts of the present invention may be used in combination with other drugs that are used in the treatment of the diseases or conditions for which the present salts are useful, e.g., treatment of obesity and/or type 2 diabetes. Such other  
5 drug(s) may be administered, by a route and in an amount commonly used therefore, contemporaneously or sequentially with a salt of the present invention. When a salt of the present invention is used contemporaneously with one or more other drugs, a pharmaceutical unit dosage form containing such other drugs in addition to the present salt is preferred. Accordingly, the pharmaceutical compositions of the present invention  
10 include those that also contain one or more other active ingredients, in addition to a salt of the present invention.

A preferred combination therapy for the treatment of obesity is the use of a salt of the present invention in combination with sibutramine (or active metabolites of sibutramine, *e.g.*, desmethyl sibutramine and di-desmethyl sibutramine), preferably with  
15 sibutramine hydrochloride mono-hydrate.

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We claim:

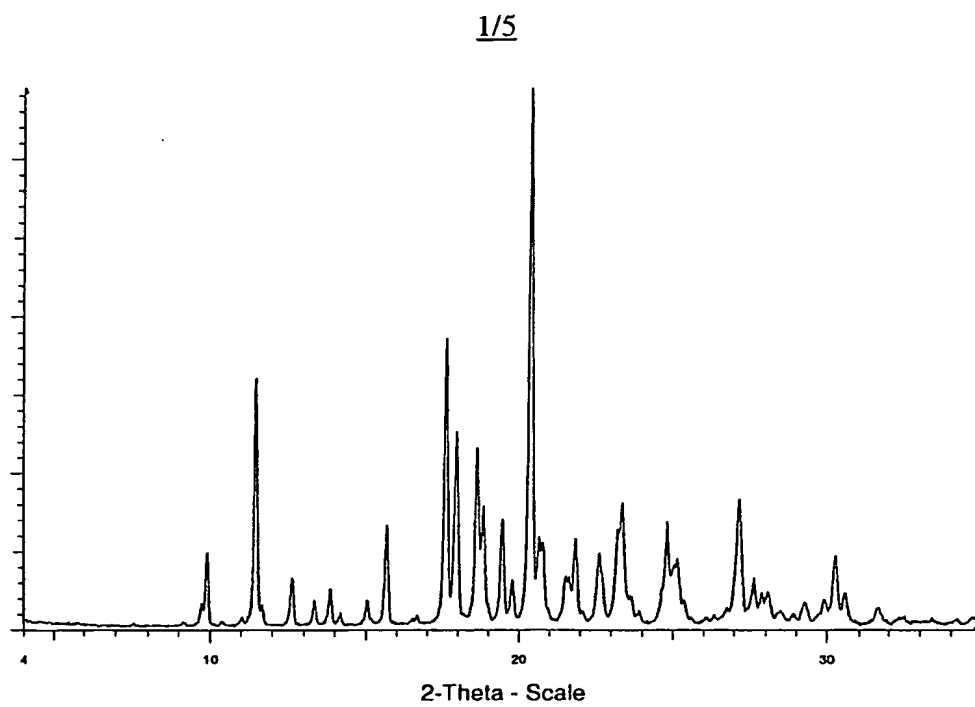
1. A crystalline pharmaceutical acid addition salt of 2-(4-{2-[2-hydroxy-3-(2-thiophen-2-yl-phenoxy)-propylamino]-2-methyl-propyl}-phenoxy)-nicotinonitrile.  
5
2. The salt of claim 1 which is the non-solvated hemi-fumarate.
3. The hemi-fumarate of claim 2 having an X-ray diffraction pattern which  
10 comprises the following peaks:  $11.4 \pm 0.1$ ,  $17.6 \pm 0.1$ ,  $17.9 \pm 0.1$  and  $20.3 \pm 0.1^\circ$  in  $2\theta$ ; when the pattern is obtained from a copper radiation source ( $\lambda = 1.54056$ ).
4. The hemi-fumarate of Claim 3 wherein said X-ray diffraction pattern  
15 further comprises the following peaks:  $18.6 \pm 0.1$ ,  $18.8 \pm 0.1$ ,  $19.4 \pm 0.1$  and  $27.1 \pm 0.1^\circ$  in  $2\theta$ .
5. The salt of claim 1 which is the hemi-fumarate hemi-hydrate.
6. The hemi-hydrate of claim 5 having an X-ray diffraction pattern which  
20 comprises the following peaks:  $11.4 \pm 0.1$ ,  $12.7 \pm 0.1$ ,  $18.6 \pm 0.1$  and  $21.3 \pm 0.1^\circ$  in  $2\theta$ ; when the pattern is obtained from a copper radiation source ( $\lambda = 1.54056$ ).
7. The hemi-hydrate of claim 6 wherein said X-ray diffraction pattern further  
25 comprises the following peaks:  $8.4 \pm 0.1$ ,  $9.9 \pm 0.1$ ,  $15.2 \pm 0.1$  and  $23.8 \pm 0.1^\circ$  in  $2\theta$ .
8. The hemi-hydrate of claim 6 or 7 wherein said X-ray diffraction pattern  
30 further comprises the following peaks:  $4.2 \pm 0.1$  and  $7.8 \pm 0.1^\circ$  in  $2\theta$ .
9. The salt of claim 1 which is the non-solvated benzoate.

- 5           10.    The benzoate of claim 9 having an X-ray diffraction pattern which comprises the following peaks:  $8.6 \pm 0.1$ ,  $14.9 \pm 0.1$ ,  $19.3 \pm 0.1$  and  $22.2 \pm 0.1^\circ$  in  $2\theta$ ; when the pattern is obtained from a copper radiation source ( $\lambda = 1.54056$ ).
- 10           11.    The benzoate of Claim 10 wherein said X-ray diffraction pattern further comprises the following peaks:  $17.2 \pm 0.1$ ,  $17.6 \pm 0.1$ ,  $18.7 \pm 0.1$  and  $20.4 \pm 0.1^\circ$  in  $2\theta$ .
- 15           12.    The salt of claim 1 which is the non-solvated (R)-mandalate.
13.    The mandalate of claim 12 having an X-ray diffraction pattern which comprises the following peaks:  $4.7 \pm 0.1$ ,  $13.2 \pm 0.1$ ,  $21.1 \pm 0.1$  and  $21.8 \pm 0.1^\circ$  in  $2\theta$ ; when the pattern is obtained from a copper radiation source ( $\lambda = 1.54056$ ).
- 20           14.    The mandalate of Claim 13 wherein said X-ray diffraction pattern further comprises the following peaks:  $16.9 \pm 0.1$ ,  $18.2 \pm 0.1$ ,  $18.6 \pm 0.1$  and  $20.0 \pm 0.1^\circ$  in  $2\theta$ .
15.    The salt of claim 1 which is the non-solvated salicylate.
- 25           16.    The salicylate of claim 15 having an X-ray diffraction pattern which comprises the following peaks:  $14.6 \pm 0.1$ ,  $16.9 \pm 0.1$ ,  $18.0 \pm 0.1$  and  $22.6 \pm 0.1^\circ$  in  $2\theta$ ; when the pattern is obtained from a copper radiation source ( $\lambda = 1.54056$ ).
- 30           17.    The salicylate of Claim 16 wherein said X-ray diffraction pattern further comprises the following peaks:  $6.9 \pm 0.1$ ,  $8.2 \pm 0.1$ ,  $8.8 \pm 0.1$  and  $19.0 \pm 0.1^\circ$  in  $2\theta$ .

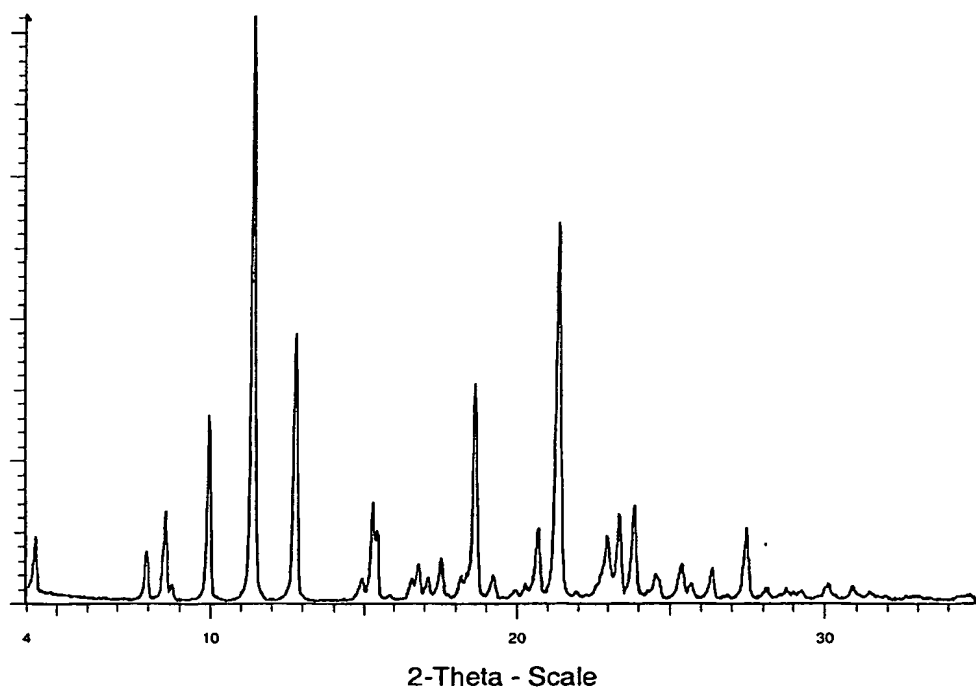
-30-

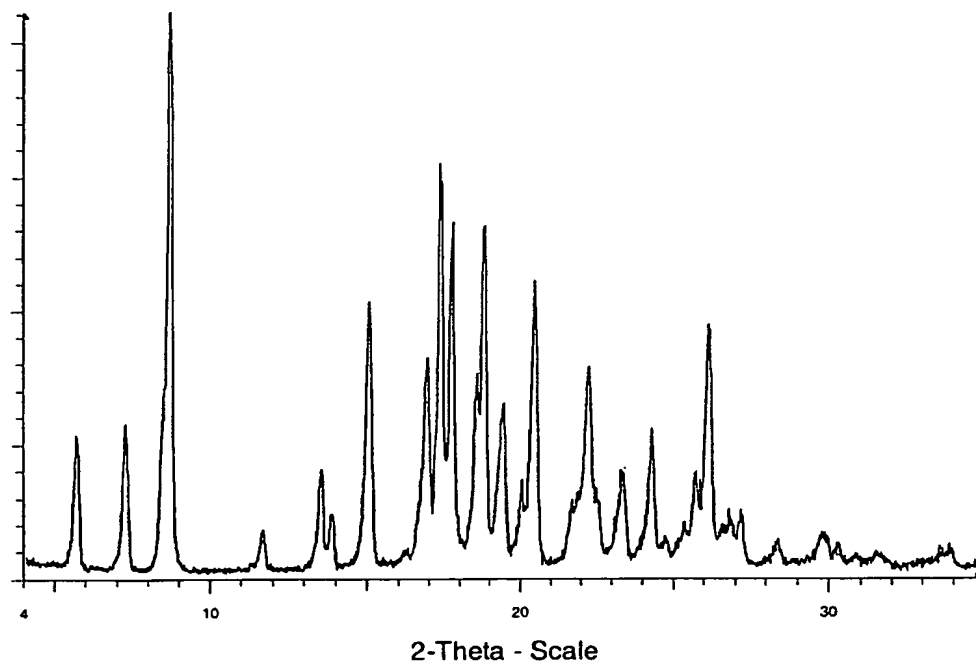
18. A pharmaceutical composition comprising a salt of any one of claims 1-17 and a pharmaceutical carrier.
- 5 19. A method of agonizing the  $\beta_3$  receptor comprising administering to a patient in need thereof an effective amount of a salt of any one of claims 1-17.
- 10 20. A method of treating obesity comprising administering to a patient in need thereof an effective amount of a salt of any one of claims 1-17.
21. A method of treating type 2 diabetes comprising administering to a patient in need thereof an effective amount of a salt of any one of claims 1-17.
- 15 22. A method of treating hypertension comprising administering to a patient in need thereof an effective amount of a salt of any one of claims 1-17.
23. The salt of any one of claims 1-17 for use in treating type 2 diabetes, obesity or hypertension or for use in agonizing the  $\beta_3$  receptor.

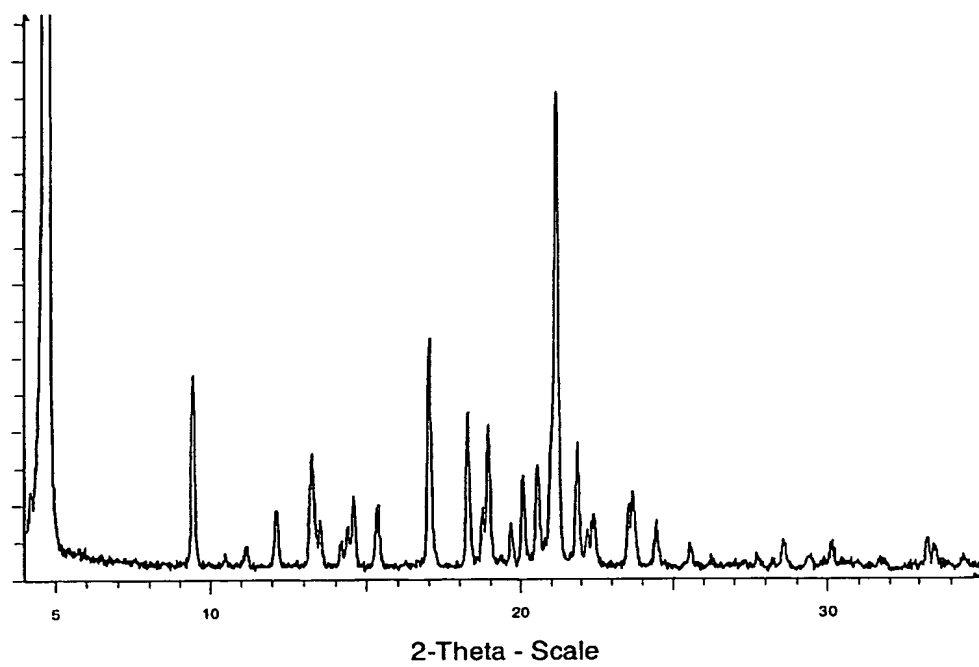
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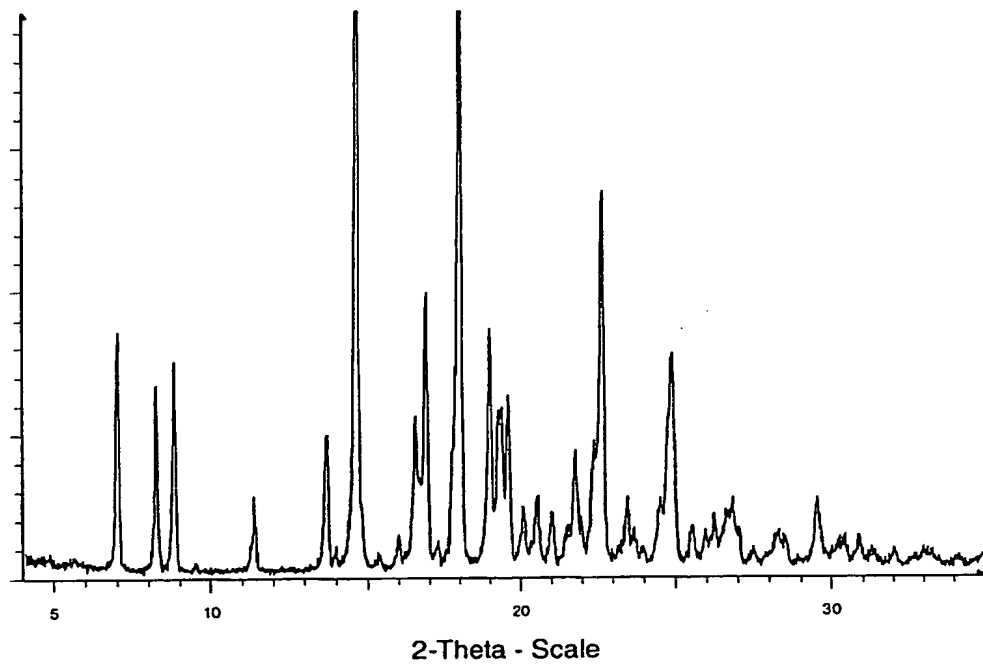




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**A. CLASSIFICATION OF SUBJECT MATTER**  
 IPC 7 C07D409/12 A61K31/44 //(C07D409/12,333:00,213:00)

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07D A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, CHEM ABS Data

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 236 624 A (BEECHAM GROUP PLC) 16 September 1987 (1987-09-16) the whole document ---	1-23
A	US 5 977 154 A (RITO CHRISTOPHER JOHN ET AL) 2 November 1999 (1999-11-02) the whole document ---	1-23
A	EP 0 678 511 A (SANKYO CO) 25 October 1995 (1995-10-25) the whole document -----	1-23

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

\* Special categories of cited documents:

\*A\* document defining the general state of the art which is not considered to be of particular relevance

\*E\* earlier document but published on or after the international filing date

\*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

\*O\* document referring to an oral disclosure, use, exhibition or other means

\*P\* document published prior to the international filing date but later than the priority date claimed

\*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

\*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

\*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

\* & \* document member of the same patent family

Date of the actual completion of the international search

19 August 2002

Date of mailing of the international search report

27/08/2002

Name and mailing address of the ISA

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Authorized officer

Frelon, D

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US 02/11896

## Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
  
Although claims 19-22 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

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